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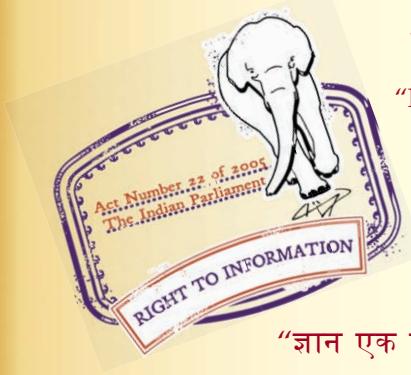
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IS 326-14 (1985): Methods of sampling and test for natural and synthetic perfumery materials (Part 14) Determination of heavy metals [PCD 18: Natural and Synthetic Fragrance Materials]

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*Indian Standard*

**METHODS OF SAMPLING AND TEST FOR  
NATURAL AND SYNTHETIC  
PERFUMERY MATERIALS**

**PART 14 DETERMINATION OF HEAVY METALS**

*( Second Revision )*

UDC 665.572/574 : 543.422 [ 546.1 ]



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**INDIAN STANDARDS INSTITUTION  
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG  
NEW DELHI 110002**

## *Indian Standard*

# METHODS OF SAMPLING AND TEST FOR NATURAL AND SYNTHETIC PERFUMERY MATERIALS

## PART 14 DETERMINATION OF HEAVY METALS

( *Second Revision* )

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## *Indian Standard*

# METHODS OF SAMPLING AND TEST FOR NATURAL AND SYNTHETIC PERFUMERY MATERIALS

## PART 14 DETERMINATION OF HEAVY METALS

### ( *Second Revision* )

## 0. F O R E W O R D

**0.1** This Indian Standard ( Second Revision ) was adopted by the Indian Standards Institution on 15 February 1985, after the draft finalized by the Natural and Synthetic Perfumery Materials Sectional Committee had been approved by the Petroleum, Coal and Related Products Division Council.

**0.2** This standard was first published in 1952 entitled as 'Methods of test for essential oils' and subsequently revised in 1968 as 'Methods of sampling and test for natural and synthetic perfumery materials'. Taking cognizance of the need to incorporate instrumental methods of analysis now in vogue and to facilitate inclusion of additional test methods or changes in the existing test methods and also to align these test methods with the corresponding ISO Standards, the Committee concerned decided to revise and split the standard and publish individual test methods as separate parts of the original standard.

**0.2.1** In order to facilitate proper reference of the method of test given in this revised part of IS : 326, Amendment No. 5 is being issued separately to the relevant clause of IS : 326-1968\*.

**0.3** Heavy metals are often present as impurities in perfumery materials. It is especially important that materials be free from such impurities, if they are to be used for medicinal purposes or in foodstuffs. Furthermore, the presence of heavy metals, in perfumery materials will often cause discolouration in such products as soap and cosmetics.

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\*Methods of sampling and test for natural and synthetic perfumery materials (*first revision* ).

**0.4** In the preparation of this standard considerable assistance has been derived from the following publications:

EOA 1 Determination of Heavy Metals, published by Essential Oil Association of USA, New York, 1967.

BS 2073 : 1976 Methods of Test for Essential Oils, published by British Standards Institution, UK.

**0.5** In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960\*.

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## 1. SCOPE

**1.1** This standard prescribes the method for determination of heavy metals, namely, copper or lead present in natural and synthetic perfumery materials by using an absorptiometric method.

## 2. DETERMINATION OF HEAVY METALS

### 2.1 Determination of Copper or Lead Present in Small Amount

**2.1.1** Place in a test tube 10 ml of the perfumery material and add an equal volume of distilled water to which one drop of concentrated hydrochloric acid has been added. Shake thoroughly and then pass hydrogen sulphide through the mixture until it is saturated. Carry out simultaneously a blank determination to which no hydrogen sulphide is passed. In the absence of heavy metals, like copper or lead no darkening in colour in either the material or the water layer is produced. A comparison of the colours of the blank and of the determination will aid in establishing the absence of heavy metals, or the presence of traces. The formation of a scum at the surface between the material and the water layers is no indication of the presence of heavy metals, unless the scum is dark in colour. If the dark colour scum is obtained, follow the procedure as given in 2.2.

### 2.2 Determination of Lead Content

**2.2.0** *Outline of the Method* — Volatile components of the perfumery material are removed by boiling a suspension in dilute hydrochloric acid followed by extraction with carbon tetrachloride. The lead in the aqueous acid solution is then determined absorptiometrically as lead dithizonate.

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\*Rules for rounding off numerical values ( revised ).

## 2.2.1 Reagents

**NOTE** — The majority of the reagents required for this determination are available commercially as 'lead-free' reagents and these are indicated below; otherwise prepare the 'lead-free' reagent by the stated procedure. Prepare the solution freshly and filter if necessary.

**2.2.1.1 Carbon tetrachloride — lead-free.**

**2.2.1.2 Chloroform** — Shake 250 ml of chloroform with 25 ml of water containing 1 ml of the potassium cyanide solution and about 20 drops of aqueous ammonia (5 M). Separate and reject the aqueous layer, wash the chloroform with water and filter it.

**2.2.1.3 Aqueous ammonia** — of density 0.880, lead-free.

**2.2.1.4 Hydrochloric acid** — (see IS : 265-1976\*), lead-free.

**2.2.1.5 Nitric acid solution** — Dilute 1 volume of nitric acid (lead-free) to 100 volumes with water (see IS : 264-1976†).

**2.2.1.6 Ammonium citrate solution** — ( $(\text{NH}_4)_3\text{C}_6\text{H}_5\text{O}_7$ ), lead-free. Prepare as follows:

Dissolve 125 g of ammonium citrate in 400 to 450 ml of water, make faintly alkaline to litmus paper with lead-free aqueous ammonia (5 M) and extract with chloroform and appropriate additions of the stock dithizone solution. Continue extraction until all metals have been removed and the extract is faintly green, then make the solution just acidic by adding lead-free hydrochloric acid (5 M), and extract with further portions of chloroform until the final extract is colourless.

**2.2.1.7 Sodium sulphite solution** — Prepare by dissolving 4 g of sodium sulphite heptahydrate ( $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$ ) in water and diluting to 100 ml.

**2.2.1.8 Ammonical sulphite-cyanide solution** — Mix 340 ml of the aqueous ammonia, 75 ml of the sodium sulphite solution, and 30 ml of the potassium cyanide solution and 605 ml of water.

**2.2.1.9 Dithizone stock solution** — 0.1 percent (m/v) solution of dithizone in chloroform. Filter and store in a refrigerator. Discard after 14 days.

**2.2.1.10 Dithizone solution** — Prepare by shaking 6 ml of the stock dithizone solution with 9 ml of water and one ml of ammonia. Separate and reject the lower layer. Centrifuge the aqueous layer until clear.

**NOTE** — Prepare a fresh solution on the day of use.

\*Specification for hydrochloric acid (second revision).

†Specification for nitric acid (second revision).

**2.2.1.11 Potassium cyanide solution** — lead-free, containing 100 g/l.

**Caution — Highly Poisonous.** Do not measure solution containing cyanide by means of a pipette filled by suction with the mouth; use a burette or other dispensing device.

**2.2.1.12 Hydroxylammonium chloride solution** — containing 200 g/l.

**2.2.1.13 Sodium hexametaphosphate solution** — lead-free. Prepare as follows:

Dissolve 10 g of sodium hexametaphosphate in 90 ml of water, adjust to pH 9 with thymol blue indicator solution by adding ( lead-free ) aqueous ammonia and extract with dithizone in chloroform until free from lead. Make the solution just acidic and remove the dithizone traces by extraction with chloroform. Finally adjust to the maximum blue colour of the indicator.

**2.2.1.14 Standard lead solution** — Dissolve 1.60 g of lead nitrate [  $\text{Pb}(\text{NO}_3)_2$  ] in water, add 10 ml of nitric acid, and dilute to 1 000 ml. Further dilute one volume of this solution freshly as required to 100 volumes with water. One ml of this solution is equivalent to 10  $\mu\text{g}$  of lead.

**2.2.1.15 Thymol blue indicator solution** — Prepare as follows:

Warm 0.1 g of thymol blue with 4.3 ml of 0.05 N sodium hydroxide solution and 5 ml of 90 percent ( v/v ) ethanol; after solution is complete, make up the volume to 250 ml with ethanol 20 percent ( v/v ).

## 2.2.2 Apparatus

**2.2.2.1 Separating funnel** — 100 and 250 ml capacity.

**2.2.2.2 Photoelectric absorptiometer** — with 0.5 cm or 1 cm cells, and a filter having a maximum transmission at 520 nm, or spectrophotometer set at 520 nm.

## 2.2.3 Procedure

**NOTE** — Atmospheric dust normally contains substantial amounts of lead and particular attention is therefore, necessary to avoid dust contamination at all stages of the determination.

**2.2.3.1** Weigh, to the nearest 0.01 g, approximately 5 g of the sample into a 250 ml beaker. Add 80 ml of water and 10 ml of hydrochloric acid. Boil gently with constant stirring for 10 minutes to volatilize most of the materials. Cool and transfer the acid solution to a 250-ml separating funnel. Add 10 ml of the carbon tetrachloride to the beaker, boil, cool and transfer to the separating funnel. Repeat this procedure, then rinse the beaker twice with 10 ml of water, adding the washings to the separating funnel. Extract with 20 ml portions of carbon tetrachloride.

until no further colour is extracted. Filter the aqueous layer through a wetted filter paper into a 100-ml volumetric flask, rinsing out the separating funnel and filter with water until 100 ml of filtrate is obtained.

**2.2.3.2** Transfer 10 ml of this solution to a small beaker, add 5 ml of ammonium citrate solution and 10 ml of sodium hexametaphosphate solution, a few drops of thymol blue indicator solution and sufficient aqueous ammonia to give a blue-green colour indicating  $\text{pH}$  9.0 to 9.5. Cool, add one ml of potassium cyanide solution, one ml of hydroxylammonium chloride solution and adjust the alkalinity to  $\text{pH}$  9.0 to 9.5, if necessary. Transfer to a 100-ml separating funnel containing 10 ml of the chloroform, washing in with a few millilitres of water. The volume of the aqueous layer at this stage should be about 50 ml. Add 0.5 ml of dithizone working solution, shake vigorously for one minute and allow to separate. If the lower layer is red, add dithizone working solution until, after shaking, a purple blue or green colour is obtained. Run the chloroform layer into a second separating funnel and rinse the first separating funnel through with one ml or 2 ml of chloroform. Add, to the liquid in the first separating funnel, 3 ml of chloroform and 0.2 ml of dithizone working solution, shake vigorously for 30 seconds, allow the chloroform layer to separate and add it to the main chloroform extract. The last chloroform extract should be green. If it is not, carry out further extractions with chloroform and dithizone working solution until the green colour of the final extract indicates that all the lead has been extracted. Alternatively, repeat the extractions using a smaller portion of the test solution. Reject the aqueous layer. Shake the chloroform extract vigorously for one minute with 10 ml of nitric acid solution. Separate, discarding the chloroform layer as completely as possible.

**2.2.3.3** Add 30 ml of the ammoniacal sulphite-cyanide solution, exactly 10 ml of chloroform and 0.5 ml of dithizone working solution, and shake the mixture vigorously for one minute. Allow it to settle. If the supernatant liquid is not pronounced orange-yellow, add a further 0.5 ml of dithizone working solution, shake the mixture again for one minute and allow it to settle. Run off a few drops of the chloroform layer, insert a plug of cotton wool into the dry stem of the funnel and, after rejecting the first runnings, fill a one-cm cell with the filtered chloroform solution. Measure the optical density of the solution against chloroform and determine the lead content from a standard curve obtained with 0, 1.0, 2.0, 3.0 and 4.0 ml of the standard lead solution, diluted to a volume of 10 ml with nitric acid solution, using the same procedure. Measure the optical density of the test solution against chloroform.

**2.2.3.4 Reagent blank determination** — Carry out a blank determination as prescribed in **2.2.3.1** to **2.2.3.3** but omitting the perfumery material.

**2.2.4 Expression of Results** — Deduct the value of the optical density for the reagent blank from that for the test solution and determine from the calibration graph the lead content of the perfumery material.

**2.2.4.1** Express the result as milligrams of lead per kilogram of the perfumery material.

### **2.3 Determination of Iron**

#### **2.3.1 Apparatus**

**2.3.1.1** *Nessler cylinders* — 100-ml capacity ( see IS : 4161-1967\* ).

#### **2.3.2 Reagents**

**2.3.2.1** *Hydrochloric acid* — 2 N ( see IS : 265-1976† ).

**2.3.2.2** *Ammonium persulphate*

**2.3.2.3** *Potassium thiocyanate solution* — approximately 5 percent.

**2.3.2.4** *Standard iron solution* — Dissolve 0.702 g of ferrous ammonium sulphate [  $\text{FeSO}_4 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$  ] in 100 ml distilled water, add 10 ml of concentrated sulphuric acid and dilute with water to 1 000 ml mark. Transfer 100 ml of this solution to a 1 000 ml volumetric flask and dilute up to the mark. One millilitre of this solution is equivalent to 0.01 mg of iron ( as Fe ).

**2.3.3 Procedure** — Add 25 ml of 2 N hydrochloric acid to 25 ml of perfumery compound, shake and boil slowly for about 10 minutes. Cool and separate the aqueous layer. Add about 30 mg of ammonium persulphate and boil to oxidize the iron. Cool and transfer to a Nessler cylinder, add 2 ml of potassium thiocyanate solution and dilute to 100 ml mark with water. In another Nessler cylinder, take the same amounts of hydrochloric acid, ammonium persulphate and potassium thiocyanate solution and dilute to about 90 ml. From a burette add standard iron solution in small portions so that after dilution to 100 ml, the colour obtained matches with that obtained with the material. The iron content of the perfumery material is then equal to the amount of iron added to obtain equivalent colouration as produced by sample solution.

### **2.4 Determination of Arsenic**

#### **2.4.1 Reagents**

**2.4.1.1** *Hydrochloric acid* — 2 N ( see IS : 265-1976† ).

**2.4.2 Procedure** — Add 25 ml of 2 N hydrochloric acid to 25 ml of perfumery compound, shake and boil slowly for about 10 minutes. Cool and separate the aqueous layer. Carry out determination of arsenic as described in 5.1 of IS : 2088-1983‡.

\*Specification for Nessler cylinders.

†Specification for hydrochloric acid ( second revision ).

‡Methods for determination of arsenic ( second revision ).

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# INTERNATIONAL SYSTEM OF UNITS ( SI UNITS )

## Base Units

QUANTITY	UNIT	SYMBOL
Length	metre	m
Mass	kilogram	kg
Time	second	s
Electric current	ampere	A
Thermodynamic temperature	kelvin	K
Luminous intensity	candela	cd
Amount of substance	mole	mol

## Supplementary Units

QUANTITY	UNIT	SYMBOL
Plane angle	radian	rad
Solid angle	steradian	sr

## Derived Units

QUANTITY	UNIT	SYMBOL	DEFINITION
Force	newton	N	1 N = 1 kg.m/s <sup>2</sup>
Energy	joule	J	1 J = 1 N.m
Power	watt	W	1 W = 1 J/s
Flux	weber	Wb	1 Wb = 1 V.s
Flux density	tesla	T	1 T = 1 Wb/m <sup>2</sup>
Frequency	hertz	Hz	1 Hz = 1 c/s (s <sup>-1</sup> )
Electric conductance	siemens	S	1 S = 1 A/V
Electromotive force	volt	V	1 V = 1 W/A
Pressure, stress	pascal	Pa	1 Pa = 1 N/m <sup>2</sup>



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